

Supplementary Information for:

mRNA Vaccines Induce Durable Immune Memory to SARS-CoV-2 and Variants of Concern

Goel, Painter, Apostolidis, Mathew, et al.

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Figure S1

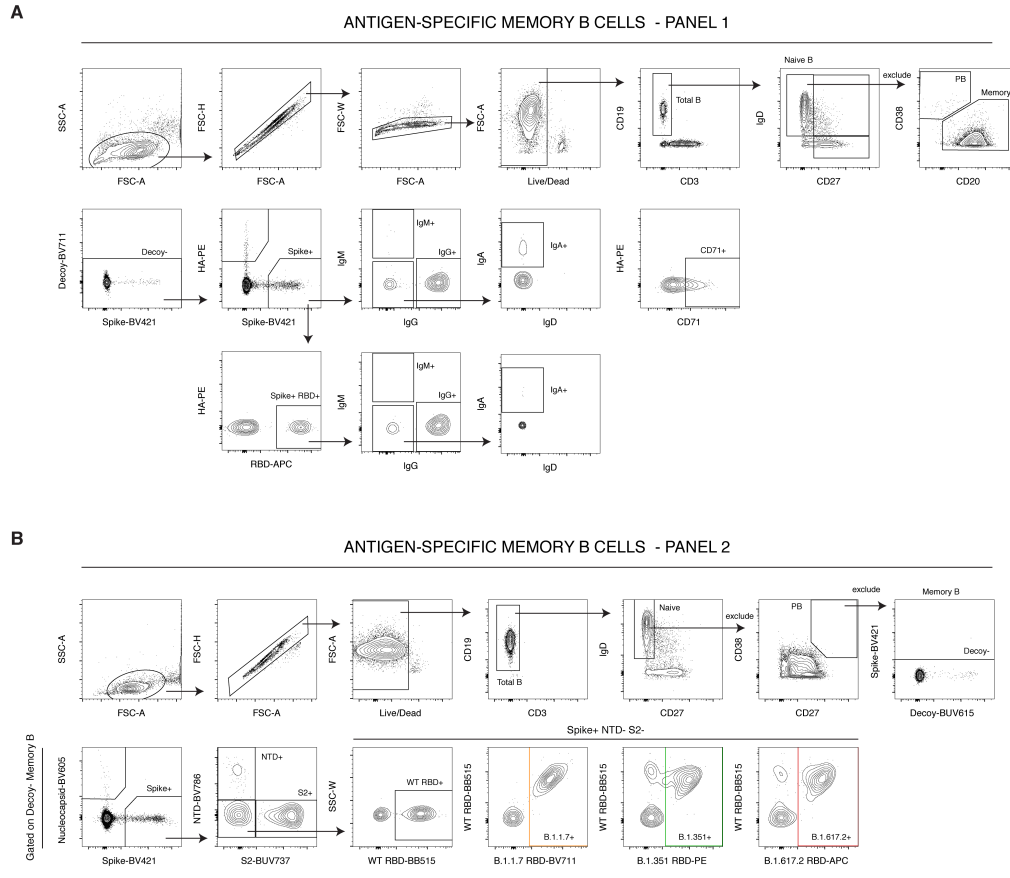


Figure S1. Gating strategy for SARS-CoV-2-specific memory B cells. **A)** For **panel 1**, lymphocytes were first identified based on forward- and side-scatter from bulk PBMC samples. Singlets were excluded by FSC-A/FSC-H and FSC-A/FSC-W. Dead cells were excluded using Ghost 510 viability dye. Total B cells were then identified as CD3- CD19+ cells. Naïve B cells were identified as IgD+ CD27- B cells and excluded from downstream analysis. Memory B cells were subsequently identified from non-naïve B cells as CD20+ CD38lo/int cells. A BV711 decoy probe was used to gate out memory B cells that non-specifically bound streptavidin. Spike- and HA-binding were then quantified on decoy- memory B cells. Binding to RBD probe was also measured on Spike+ memory B cells. IgG, IgM, and IgA isotypes were evaluated for both Spike+ and Spike+ RBD+ memory B cells. CD71 was measured as an activation marker on Spike+ memory B cells. **B)** For **panel 2**, total B cells were enriched by negative selection from PBMC samples prior to staining. Live, non-naïve B cells were identified as described above. Plasmablasts were then identified as CD27+ CD38+ non-naïve B cells and were excluded from downstream analysis. Decoy-cells were excluded as described above. Spike- and nucleocapsid-specific B cells were identified based on binding to corresponding probes. Spike+ memory cells were then analyzed for co-binding to N-terminal domain (NTD) or S2 domain probes. Memory B cells that were Spike+ but NTD- and S2- were subsequently analyzed for co-binding to a panel of variant RBD probes, including wild-type (WT), B.1.1.7, B.1.351, and B.1.617.2 RBDs. IgG expression was evaluated for all antigen-specific populations.

Figure S2

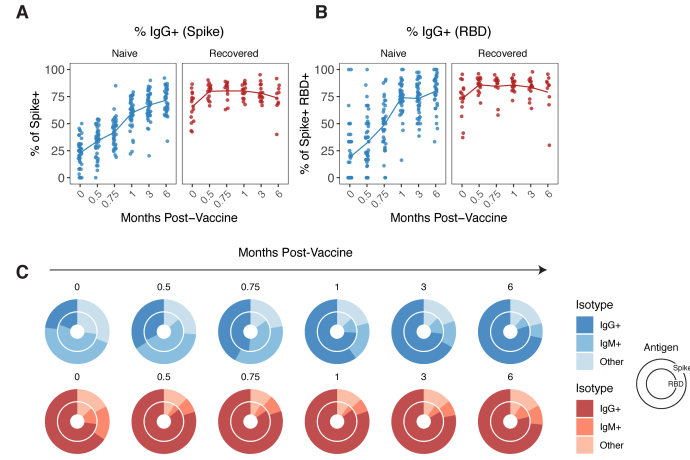


Figure S2. Class-switching of SARS-CoV-2-specific memory B cells after mRNA vaccination. A) Percent IgG+ of Spike+ and **B)** Spike+ RBD+ memory B cells over time after mRNA vaccination. Lines connect mean values at different timepoints. **C)** Summary statistics for % IgG+, % IgM+, and % other isotype+ of SARS-CoV-2-specific memory B cells over time. Outer rings represent total Spike+ memory B cells, inner rings represent Spike+ RBD+ memory B cells.

Figure S3

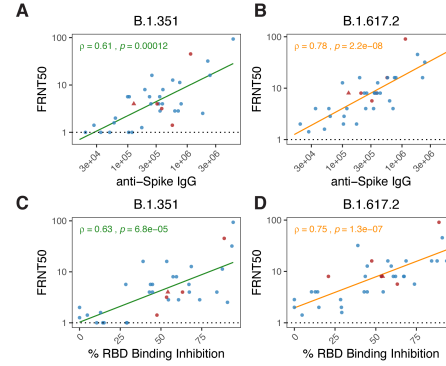


Figure S3. Functional characterization of memory B cell-derived SARS-CoV-2-specific antibodies. A) Correlation of anti-Spike IgG with pseudovirus neutralization titers against B.1.351 and B) B.1.617.2 in culture supernatants from *in vitro* stimulation. C) Correlation of hACE2-RBD-binding inhibition with pseudovirus neutralization titers against B.1.351 and D) B.1.617.2 in culture supernatants from *in vitro* stimulation. Correlations were calculated using non-parametric Spearman rank correlation.

[illegible]

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Figure S5

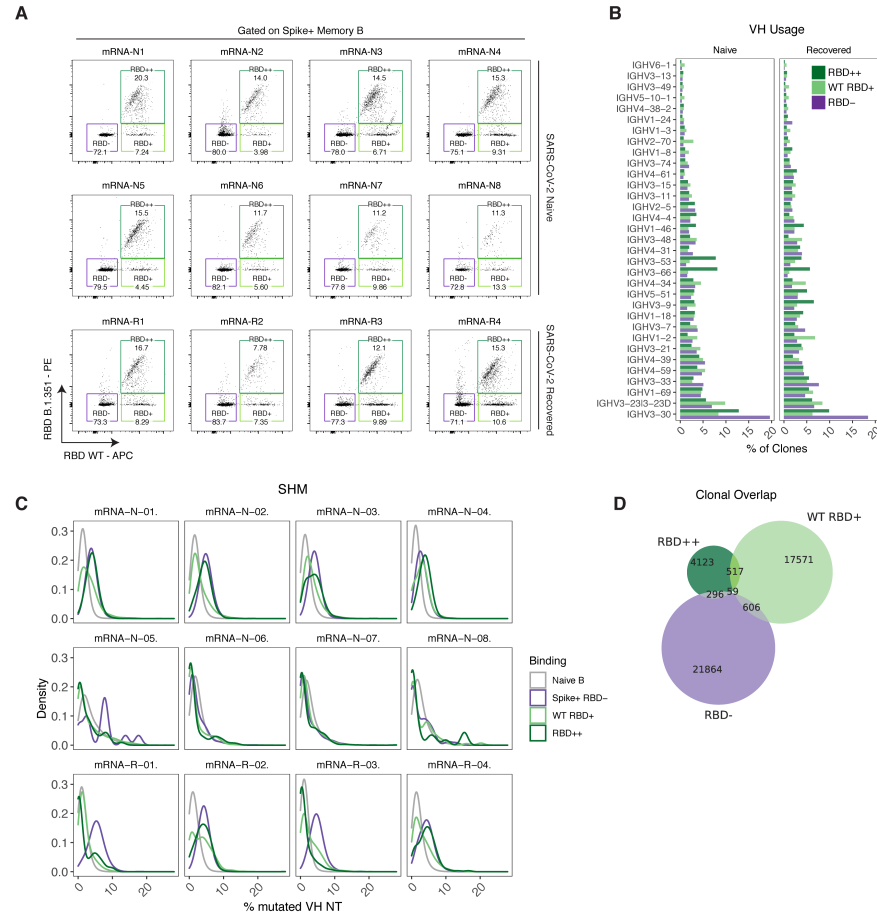


Figure S5. Extended analysis of SARS-CoV-2-specific antibody sequences. **A)** Individual flow plots of sorted memory B cell populations in 8 SARS-CoV-2 naïve and 4 SARS-CoV-2 recovered individuals. **B)** VH gene usage across different antigen binding populations. Data are represented as a percentage of the overall clones for a given antigen-binding population. **C)** Individual somatic hypermutation (SHM) distributions of memory B cell clones for SARS-CoV-2 naïve and recovered subjects. Data are represented as the percent of mutated VH gene nucleotides. **D)** Venn diagram of clonal overlap between RBD-, WT RBD+ and RBD++ populations. Data were filtered based on larger clones with $\geq 50\%$ mean copy number frequency in each sequencing library.

Figure S6

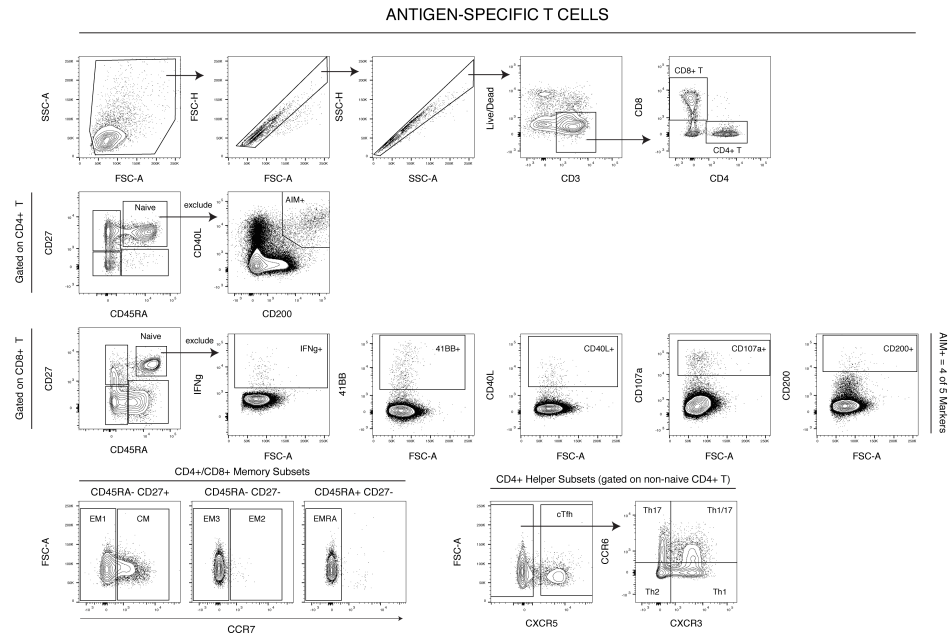


Figure S6. Gating strategy for SARS-CoV-2-specific memory T cells. Lymphocytes were first identified based on forward- and side-scatter from bulk PBMC samples. Singlets were identified by FSC-A/FSC-H and SSC-A/SSC-H. Total T cells were identified as Live/Dead- CD3+. CD4 and CD8 T cells were then identified from total T cells. For both CD4+ and CD8+ T cells, naïve cells were identified as CD45RA+ CD27+ and excluded from downstream analysis. Memory subsets were defined based on a combination of CD45A, CD27, and CCR7 expression. CD4+ helper subsets were defined based on CCR6, CXCR3, and CXCR5 chemokine receptor expression. AIM+ CD4+ T cells were identified based on co-expression of CD40L and CD200. AIM+ CD8+ T cells were identified based on co-expression of at least 4 of 5 activation induced markers (intracellular IFN-g, 41BB, CD40L, CD107a, CD200).

Figure S7

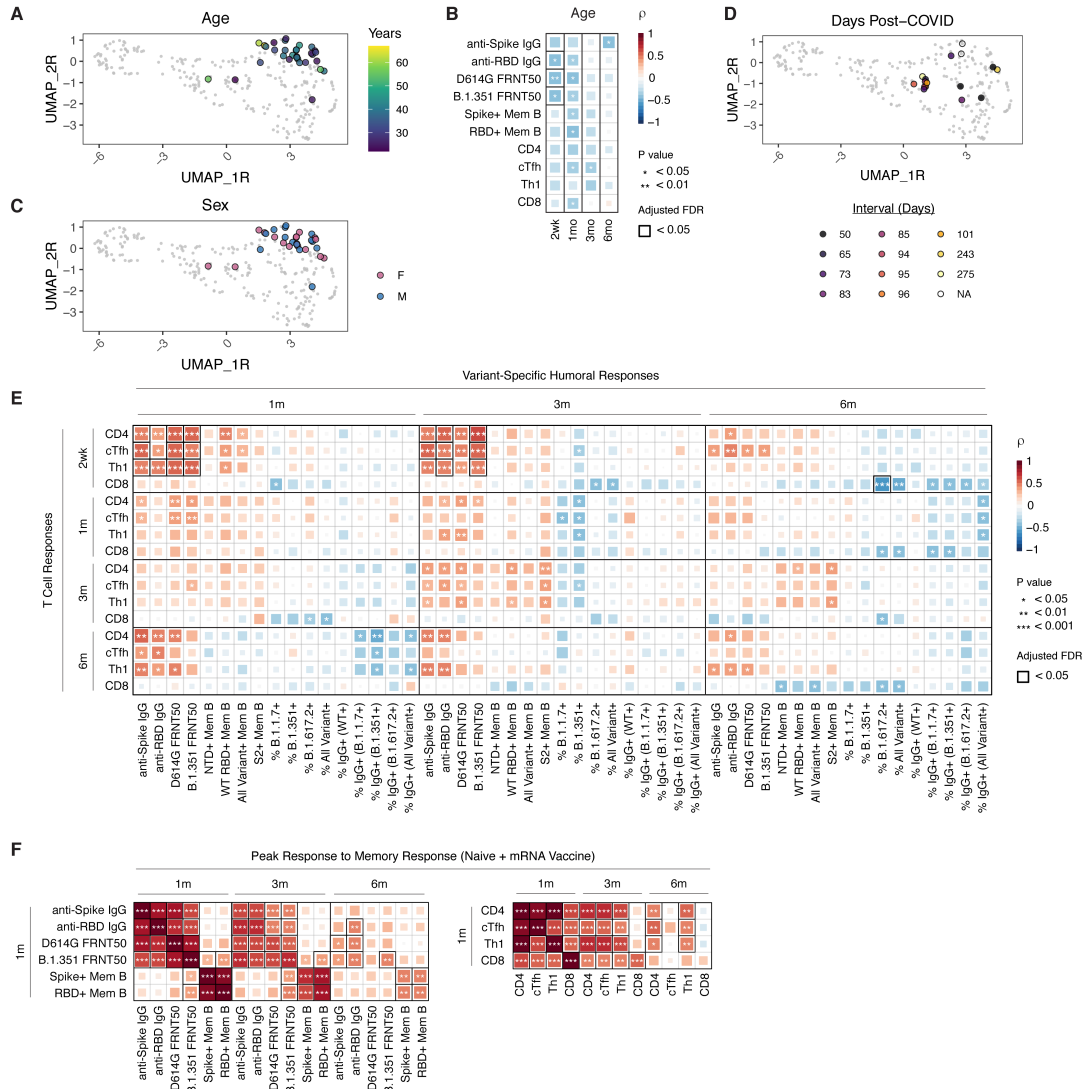


Figure S7. Immune correlations after mRNA vaccination. **A)** Relationship between age and overall vaccine response in SARS-CoV-2 naïve subjects. 6-month post-vaccination samples are colored by age and projected onto the UMAP coordinates from figure 6. **B)** Correlation between individual immune parameters and age over time after vaccination in SARS-CoV-2 naïve subjects. **C)** Relationship between sex and overall vaccine response in SARS-CoV-2 naïve subjects. 6-month post-vaccination samples are colored by sex and projected onto the UMAP coordinates from figure 6. **D)** Relationship between time since infection and pre-vaccine immune phenotype in SARS-CoV-2 recovered subjects. Pre-vaccine samples are colored based on days since infection and projected onto the UMAP coordinates from figure 6. **E)** Correlation between T cell responses and variant-specific humoral responses over time in SARS-CoV-2 naïve subjects. **F)** Correlation between peak antibody, memory B, and memory T cell responses 1 week after the second vaccine dose with later responses at 3- and 6-months post-vaccination in SARS-CoV-2 naïve subjects. All statistics were calculated using non-parametric Spearman rank correlation.

		SARS-CoV-2 Naïve	SARS-CoV-2 Recovered	Infection Only
Total	Number	45	16	26
Age	Average (Years)	36.9	38.3	35.5
	Range (Years)	22-67	23-59	25-58
	20-30	15	4	10
	30-40	14	6	9
	40-50	9	2	4
	50+	7	4	3
Sex	Male	21	10	9
	Female	24	6	17
Race/Ethnicity	White - Non-Hispanic/Latino	27	7	23
	White - Hispanic/Latino	4	1	0
	Asian	8	6	1
	Black	3	1	2
	Native	0	1	0
	Mixed	2	0	0
	Other	1	0	0
Vaccine Type	Pfizer	42	12	--
	Moderna	3	4	--
Time Between Infection and Vaccine	Average (Days)	--	102.4	--
	Range (Days)	--	50-275; 2 N/A	--

Table S1. Demographic Information for University of Pennsylvania Healthy COVID Vaccine and Healthcare Worker (HCW) Sero-Monitoring Studies. Number of participants, age, sex, race/ethnicity, and vaccine type are indicated. For SARS-CoV-2 recovered vaccinees, time between infection and vaccine is also indicated.

Sample ID	Recovered	Population	Input DNA (ng)	Copies	Unique	# of 2-Copy Productive Clones	Average VH Identity	CDR3 Length (NT)
N1	No	Naïve B	200	122567	98404	11990	0.98	53.84
N1	No	Spike+ RBD-	35.88	148254	118969	5701	0.96	51.01
N1	No	Spike+ WT RBD+	9.516	211511	108374	1905	0.97	49.84
N1	No	Spike+ RBD++	3.614	185656	93739	1458	0.96	49.28
N2	No	Naïve B	200	137926	116872	15574	0.98	54.91
N2	No	Spike+ RBD-	46.8	126678	106225	6081	0.95	54.54
N2	No	Spike+ WT RBD+	21.19	161592	105657	3703	0.97	54.43
N2	No	Spike+ RBD++	5.746	172531	89797	1395	0.96	51.98
N3	No	Naïve B	200	144164	114631	12975	0.98	54.20
N3	No	Spike+ RBD-	20.67	142926	93621	5040	0.96	51.39
N3	No	Spike+ WT RBD+	29.9	152730	112390	5439	0.97	53.33
N3	No	Spike+ RBD++	3.692	156165	78526	1131	0.96	50.55
N4	No	Naïve B	200	171525	130359	12634	0.99	55.88
N4	No	Spike+ RBD-	46.8	178318	129650	4218	0.97	53.15
N4	No	Spike+ WT RBD+	N.D	162184	28112	215	0.97	51.56
N4	No	Spike+ RBD++	3.952	215612	87554	899	0.96	49.92
N5	No	Naïve B	200	132781	102777	17464	0.96	53.05
N5	No	Spike+ RBD-	N.D	6786	1397	12	0.93	57.00
N5	No	Spike+ WT RBD+	5.356	111474	44545	979	0.96	53.13
N5	No	Spike+ RBD++	N.D	135740	18454	139	0.97	49.47
N6	No	Naïve B	200	180913	145385	16281	0.97	52.97
N6	No	Spike+ RBD-	4.914	157476	73933	1336	0.98	51.79
N6	No	Spike+ WT RBD+	4.628	214823	75025	1016	0.98	50.56
N6	No	Spike+ RBD++	N.D	123634	32078	313	0.98	50.83
N7	No	Naïve B	200	195059	155054	18795	0.97	52.16
N7	No	Spike+ RBD-	2.392	243454	85933	944	0.98	49.51
N7	No	Spike+ WT RBD+	7.514	243014	118718	1863	0.97	50.98
N7	No	Spike+ RBD++	1.638	265908	61669	557	0.98	47.70
N8	No	Naïve B	127.4	118333	91634	11738	0.97	52.85
N8	No	Spike+ RBD-	N.D	168131	37287	251	0.97	55.02
N8	No	Spike+ WT RBD+	N.D	113831	24573	220	0.97	49.60
N8	No	Spike+ RBD++	N.D	17036	3030	62	0.97	42.92
R1	Yes	Naïve B	200	143463	110315	20150	0.99	54.68
R1	Yes	Spike+ RBD-	22.75	167823	90997	2953	0.95	52.46
R1	Yes	Spike+ WT RBD+	23.92	152168	91549	4541	0.98	55.47
R1	Yes	Spike+ RBD++	N.D	154271	30773	237	0.98	52.24
R2	Yes	Naïve B	200	130861	118251	40083	0.99	52.92
R2	Yes	Spike+ RBD-	19.63	140803	90062	3648	0.96	52.23
R2	Yes	Spike+ WT RBD+	2.964	219907	75961	872	0.96	53.12
R2	Yes	Spike+ RBD++	N.D	234281	58686	599	0.96	52.52
R3	Yes	Naïve B	200	210839	164405	26661	0.99	55.22
R3	Yes	Spike+ RBD-	22.49	122283	76751	3321	0.95	50.32
R3	Yes	Spike+ WT RBD+	7.046	239056	105423	1738	0.97	54.58
R3	Yes	Spike+ RBD++	N.D	202133	49395	418	0.99	52.24
R4	Yes	Naïve B	200	167836	134501	19019	0.98	54.34
R4	Yes	Spike+ RBD-	23.01	176440	108360	3355	0.96	52.49
R4	Yes	Spike+ WT RBD+	13.65	187951	94348	2940	0.97	52.69
R4	Yes	Spike+ RBD++	5.512	147560	53828	893	0.96	51.36

Table S2. BCR Sequencing Metadata. Sample ID indicates subject; Recovered indicates prior COVID-19. Two independent PCR amplifications (biological replicates) were performed for each sample; Total input DNA from 2 replicates; Number of valid sequence copies (passing length and other QC filters, see methods); Clones are defined as sequences that share the same VH, JH, CDR3 length and are at least 85% identical in the third complementarity determining region (CDR3) amino acid sequence; Productive rearrangements only. Clones with only 1 copy at the subject level are excluded; Average VH identity compared to the nearest germline VH gene (average identity was calculated for each clone and then averaged across clones with each clone counted once per sample); CDR3 length in nucleotides (nt).

	Fold Change at 3 Months		Decay (3-6 Months)	Estimated Duration of Boosting (Days from Vaccine)	
	Pre-Boost Baseline	Naïve Peak Response	Half-Life (Days)	Pre-Boost Baseline	Naïve Peak Response
anti-Spike IgG	12.3 (7.0 - 17.7)	0.92 (0.52 - 1.32)	48	264 (224 - 289)	84 (45 - 109)
anti-RBD IgG	10.7 (6.0 - 15.4)	0.72 (0.41 - 1.04)	47	251 (211 - 275)	68 (29 - 93)
D614G FRNT50	33.9 (14.6 - 53.3)	5.43 (2.33 - 8.53)	72	456 (368 - 503)	266 (178 - 313)
B.1.351 FRNT50	43.7 (17.4 - 70.1)	7.10 (2.83 - 11.38)	63	433 (350 - 476)	268 (185 - 311)

Table S3: Estimated Duration of Vaccine-Boosted Antibody Responses in SARS-CoV-2 Recovered Individuals. Data are presented as mean values with 95% confidence intervals in brackets. Fold change was calculated by dividing antibody levels at 3 months post-vaccination in SARS-CoV-2 recovered individuals by baseline (pre-vaccine) levels, or levels at 1-month post-vaccine in SARS-CoV-2 naïve individuals (naïve peak response). To estimate the duration of boosting (i.e., time to return to pre-vaccine baseline or naïve peak response levels), the number of times the fold change could be halved before reaching a value of 1 was multiplied by the mean calculated population half-lives for decay from 3-6 months.

Reagent	Vendor	Identifier	Concentration
Panel 1 - B Cell Probe			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10549-050	200ng
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10500-050	25ng
HA(Δ TM)(A/Brisbane/02/2018)(H1N1)	Immune Tech	IT-003-00110 Δ TMp	50ng
HA(Δ TM)(B/Colorado/06/2017)	Immune Tech	IT-003-B21 Δ TMp	50ng
BV421 Streptavidin	Biolegend	405226	20ng
BV711 Streptavidin	BD Biosciences	563262	20ng
PE Streptavidin	Biolegend	405203	16ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
BUV563 anti-CD3	BD Biosciences	748569	1:200
BV750 anti-CD19	Biolegend	302262	1:100
BUV805 anti-CD20	BD Biosciences	612905	1:500
BUV395 anti-CD27	BD Biosciences	563815	1:200
BUV661 anti-CD38	BD Biosciences	612969	1:200
APC-H7 anti-CD71	BD Biosciences	563671	1:50
FITC anti-IgA	Miltenyi	130-113-475	1:400
BV480 anti-IgD	BD Biosciences	566138	1:50
PE-Cy7 anti-IgG	Biolegend	410722	1:400
PerCP/Cy5.5 anti-IgM	Biolegend	314512	1:400
Panel 2 - Variant B Cell Probe			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	AVI10549-050	200ng
SARS-CoV-2 Biotinylated RBD	Acro Biosystems	SPD-C82E9-25ug	25ng
SARS-CoV-2 Biotinylated RBD (N501Y)	Acro Biosystems	SPD-C82E6-25ug	25ng
SARS-CoV-2 Biotinylated RBD (K417N/E484K/N501Y)	Acro Biosystems	SPD-C82E5-25ug	25ng
SARS-CoV-2 Biotinylated RBD (L452R/K478N)	Acro Biosystems	SPD-C82Ed-25ug	25ng
SARS-CoV-2 Biotinylated N-Terminal Domain	Sino Biological	40591-V49H-B	30ng
SARS-CoV-2 Biotinylated S2	Acro Biosystems	S2N-C52E8-25ug	50ng
SARS-CoV-2 Biotinylated Nucleocapsid	R&D Systems	BT10474-050	50ng
BV421 Streptavidin	Biolegend	405226	20ng
BV605 Streptavidin	Biolegend	405229	14ng
BV711 Streptavidin	BD Biosciences	563262	12.5ng
BV786 Streptavidin	BD Biosciences	563858	12ng
BUV615 Streptavidin	BD Biosciences	613013	12.5ng
BUV737 Streptavidin	BD Biosciences	612775	12ng
BB515 Streptavidin	BD Biosciences	564453	12.5ng
PE Streptavidin	Biolegend	405203	12.5ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
BUV563 anti-CD3	BD Biosciences	748569	1:200
BV750 anti-CD19	Biolegend	302262	1:100
BUV395 anti-CD27	BD Biosciences	563815	1:200
BUV661 anti-CD38	BD Biosciences	612969	1:200
BV480 anti-IgD	BD Biosciences	566138	1:50
APC-H7 anti-IgG	BD Biosciences	561297	1:100
Panel 3 - Variant-Specific B Cell Sorting			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10549-050	200ng
SARS-CoV-2 Biotinylated RBD	Sino Biological	40592-V08B-B	25ng

SARS-CoV-2 Biotinylated RBD (K417N/E484K/N501Y)	Sino Biological	40592-V08H85-B	25ng
BV421 Streptavidin	Biolegend	405226	20ng
AF488 Streptavidin	Biolegend	405235	20ng
PE Streptavidin	Biolegend	405203	12.5ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
APC-Cy7 anti-CD19	BD Biosciences	557791	1:200
BV650 anti-CD20	Biolegend	302336	1:200
BV785 anti-CD27	Biolegend	302832	1:66
PE-Cy7 anti-CD38	eBioscience	25-0389-42	1:200
PE-CF594 anti-IgD	BD Biosciences	562540	1:50

Table S4. Reagents for Memory B Cell Analysis. Reagent, vendor, catalog number, and concentration/dilution are indicated.

Reagent	Vendor	Identifier
Flow Cytometry Antibodies		
BUV395 CD4	BD Biosciences	Cat#563550
BUV496 CD8	BD Biosciences	Cat#612943
BUV615 CD45RA	BD Biosciences	Cat#751555
BUV737 CD27	BD Biosciences	Cat#612829
BUV805 CD3	BD Biosciences	Cat#612896
BV421 CXCR3	Biolegend	Cat#353716
BV650 CCR7	Biolegend	Cat#353234
BV605 CD69	Biolegend	Cat#310938
BV711 CD40L	Biolegend	Cat#310838
BV785 CD107a	Biolegend	Cat#328644
FITC IFN γ	Biolegend	Cat#502515
PE CD200	Biolegend	Cat#399804
PE-Cy7 OX40	Biolegend	Cat#350012
AF647 41BB	Biolegend	Cat#309810
APC-R700 CXCR5	BD Biosciences	Cat#565191
APC-Cy7 CCR6	Biolegend	Cat#353432
Peptides		
CD4-S peptide Megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
CD8-E peptide Megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
Other		
Ghost Dye Violet 510	Tonbo	Cat#13-0870-T500
GolgiStop (Containing Monensin)	BD Biosciences	Cat#51-2092K7
CD40 Antibody, anti-human, pure-functional grade	Miltenyi Biotech	Cat#130-094-133
Anti-Human CD28/CD49d Purified	BD Biosciences	Cat#347690
Human TruStain FcX™ (Fc Receptor Blocking Solution)	Biolegend	Cat#422302
Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent	eBioscience	Cat#00-5521-00

Table S5. Reagents for Memory T Cell Analysis. Reagent, vendor, and catalog number are indicated.